Chemico-Biological Interactions 238 (2015) 42–47

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Comparative effects of hawthorn (*Crataegus pinnatifida* Bunge) pectin and pectin hydrolyzates on the cholesterol homeostasis of hamsters fed high-cholesterol diets

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ARTICLE INFO

Article history:
Received 23 December 2014
Received in revised form 31 May 2015
Accepted 3 June 2015
Available online 10 June 2015

Keywords:
Haw pectin
Haw pectin hydrolyzates
Haw pectin pentasaccharide
Cholesterol homeostasis
Hamster

ABSTRACT

This study aims to compare the effects of feeding haw pectin (HP), haw pectin hydrolyzates (HPH), and haw pectin pentasaccharide (HPPS) on the cholesterol metabolism of hypercholesterolemic hamsters induced by high-cholesterol diets. The animals were fed a standard diet (SD), high-cholesterol diet (HCD), or HCD plus HP, HPH, or HPPS at a dose of 300 mg/kg body weight for 4 weeks. Results showed that HPPS was more effective than HP and HPH in decreasing the body weight gain (by 38.2%), liver weight (by 16.4%), and plasma and hepatic total cholesterol (TC; by 23.6% and 27.3%, respectively) of hamsters. In addition, the bile acid levels in the feces were significantly higher by 39.8% and 132.8% in the HPH and HPPS groups than in the HCD group. Such changes were not noted in the HP group. However, the HP group had higher cholesterol excretion capacities than the HPH and HPPS groups by inhibiting cholesterol absorption in the diet, with a 21.7% increase in TC excretion and a 31.1% decrease in TC absorption. Thus, HPPS could be a promising anti-atherogenic dietary ingredient for the development of functional food to improve cholesterol metabolism.

1. Introduction

Hypercholesterolemia is a key risk factor for cardiovascular disease and is positively correlated with the incidence and mortality of coronary heart disease, which is responsible for 42% of deaths in the United States [1]. Lora et al. [2] found that a 1% decrease in blood cholesterol level can lower the risk of coronary events by up to 3%. Thus, reducing high blood cholesterol level is an effective way to prevent the occurrence of these diseases.

Dietary fiber lowers blood lipid level in rats, hamsters, and mice with minimal side effects, in contrast to those frequently associated with pharmaceuticals [3–6]. Hawthorn (*Crataegus pinnatifida* Bunge) of the rose family is an excellent food source of dietary fiber. Hawthorn possesses higher pectin content than other cultivated fruits [7]. The haw pectin (HP) also exhibits four to six-fold higher viscosity than commercially available lemon and apple pectins; its molecular structure contains homogalacturonan and ramnogalacturonan main chains [8]. Pectin is a widely used food additive that functions as thickener, gelling agent, and food fiber human health supplement. Oligogalacturonic acids with degrees of polymerization (DPs) ranging from 2 to 11 were fractionated from HP hydrolyzates (HPH) through pectinase treatment [9]. HP pentasaccharide (HPPS), an α-(1-4)-D-galacturonic acid with a DP of 5, is the most abundant oligogalacturonic acid in HPH.

Compared with high-fat diet, HPPS can significantly decrease the serum and hepatic concentrations of total cholesterol (TC) and triglyceride (TG), as well as increase bile acid excretion in feces [10]. However, HP is highly viscous and is difficult to completely dissolve in water [11]. HPH contains a mixture of various oligosaccharides, including HPPS [12]. However, no study has ever explored whether or not HP and HPH display the same capacity as HPPS in improving cholesterol metabolism in high-cholesterol diet (HCD) administration. The bile composition and cholesterol metabolism of hamster are similar to those of human [13,14].

Abbreviations: HP, haw pectin; HPH, haw pectin hydrolyzates; HPPS, haw pectin pentasaccharide; SD, standard diet; HCD, high-cholesterol diet; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; NEFA, nonesterified fatty acid; ACO, acyl-CoA oxidase; CYP7A, cholesterol 7α-hydroxylase.

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http://dx.doi.org/10.1016/j.cbi.2015.06.006
0009-2797/

Thus, hamster is commonly used as an animal model to understand cholesterol metabolism. The current study compared the effects of HP, HPH, and HPPS on cholesterol metabolism and fecal bile acid excretion with hamster as the animal model. The results of this study may serve as a theoretical reference for HP utilization.

2. Materials and methods

2.1. Materials

Fresh hawthorn was purchased from a farm on the outskirts of Shenyang (Liaoning, China). HP was obtained through hot water extraction as previously described [7]. HP was dried, ground and then subjected to enzymolysis in 0.02 mmol/L acetate buffer (pH 4.0) at 45 °C with 2% substrate concentration [15]. Commercially available pectinase (DSM Food Specialties, China) at 0.2 U/mL was used to cleave HP for 4 h. The reaction solution was boiled for 10 min to destroy the enzyme activity and then centrifuged at 5000 rpm for 30 min to remove the precipitate. The supernatant was concentrated by water bath and vacuum freeze–dried to obtain HPH. The obtained HPH (10 g) was subjected to a DEAE-Sephadex A-25 column chromatograph (5.0 cm × 40 cm, HCO_3^- form) as previously described [16]. The column was directly eluted with one bed volume of 0.25 mol/L NH_4HCO_3 and then one bed volume of 0.3 mol/L NH_4HCO_3 to obtain the HPPS fraction. The HPPS fraction was deionized and vacuum freeze–dried for further analysis [9].

2.2. Animals and diets

Fifty male golden hamsters (4 weeks old) were purchased from the experimental animal center of Chinese Academy of Medical Sciences (Beijing, China). All hamsters were individually housed in stainless steel cages and initially fed a chew diet (Beijing HFK Bioscience Co., Ltd.) for 7 d. After being acclimatized to air-conditioned laboratory (22 ± 2 °C, 55% ± 5% humidity, and 12 h light/dark cycle), the hamsters were divided into five groups (n = 10 each) according to their average body weight and fed a standard diet (SD), a HCD, or a HCD containing HP (300 mg/kg body weight), HPH (300 mg/kg body weight), or HPPS (300 mg/kg body weight). The composition of the SD and HCD is shown in Table 1. All groups were treated daily with an oral infusion of HP, HPH, HPPS, or the same volume of water (SD and HCD groups). The hamsters were fed the diets for 4 weeks, and all animals had free access to food and water. The food intake and body weight of the hamsters were recorded once daily. Feces samples were collected daily and freeze–dried into powder for further analysis.

After 4 weeks of feeding followed by 12 h of food deprivation, the animals were anesthetized with isoflurane (AMRESCO, USA). Blood samples were collected through the orbital sinus, placed in EDTA K3 tubes at 37 °C for 30 min, and then centrifuged at 3000 rpm for 5 min at 4 °C. Plasma samples were stored at −80 °C for further analysis. After laparotomy, the livers were surgically removed, weighed, divided into four lobes, individually placed in sterile bags, and then immediately frozen in liquid N_2. The liver samples were stored at −80 °C for subsequent analysis of the metabolites of each experimental unit.

Table 1
Composition of the experimental diets.

Ingredients	Standard diet (%)	High-cholesterol diet (%)
Chow diet	97.6	82.6
Lard	2	10
Cholesterol	0.4	2
Yolk powder	0	5
Sodium choate	0	0.2
Propylthiouracil	0	0.2

2.3. Plasma lipid analysis

Plasma TC, high-density lipoprotein cholesterol (HDL-C), and TG levels were enzymatically determined using commercial kits from Sigma Diagnostics (St. Louis, MO, USA). Plasma non-esterified fatty acid (NEFA) was determined using the Wako NEFA C-test (Wako Pure Chemical Industries, Osaka, Japan).

2.4. Hepatic tissue morphology and lipid analysis

Livers were removed and fixed in a buffer solution of 10% formalin at room temperature. Fixed tissues were routinely processed for paraffin embedding, sliced, and then stained with hematoxylin and eosin (H&E). An i-Solution DT image acquisition and analysis program (IMT i-solution, Vancouver, Canada) incorporating a Olympus microscope (Olympus, Japan) was used for the densitometric analysis of H&E-stained tissue sections at 200× magnification. The extent of fat droplets in the liver lobule was defined as the percent of the field area within the default color range determined by the software. Data from each slide were pooled to determine means.

Hepatic total lipids were extracted with a chloroform/methanol mixture in accordance with the procedure developed by Folch et al. [17]. In brief, tissue lysates were prepared in phosphate buffer by using Ultra-Turrax® (IKA, T10 basic, Germany) until tissue lysis was complete. Lipids were extracted by mixing 125 μL of lysates with 1 mL of chloroform:methanol (2:1). The chloroform phase was evaporated under a nitrogen flux. Total liver lipids were gravimetrically quantified by evaporating the solvents in the lipid extract. The dried lipid residues were dissolved in 1 mL of ethanol for the TC and TG assays. The TC and TG levels of the extracts were determined with an enzymatic colorimetric kit (Sigma) in accordance with the procedures described by Cho et al. [18].

2.5. Fecal lipid analysis and bile acid excretion

Fecal lipids were extracted using the same method as the lipid extraction of liver samples. Fecal total lipids were gravimetrically determined by evaporating the organic solvent in the fecal lipid extracts. The TC level in the fecal lipid extract was estimated using a tissue total cholesterol assay kit (Sigma). Bile acids in the fecal samples were extracted with boiling ethanol as described by Beher et al. [19]. The total bile acid level in the extract was measured using a Total Bile Acid assay kit (BioQuant, Heidelberg, Germany). In brief, the bile acid and Thio-NADH were converted to 3-keto steroids and Thio-NADH by 3-α hydroxysteroid dehydrogenase. The change in absorbance at 405 nm was proportional to the bile acid content. Bile acid concentration was determined using a conjugated cholic acid standard in accordance with the protocol provided by the manufacturer [5].

2.6. Statistical analysis

All data are presented as mean ± SE. Data were subjected to one-way ANOVA and Duncan's multiple-range test with SPSS software. Statistical significance was considered at $p < 0.05$.

3. Results

3.1. Food intake and body and liver weights

The growth parameters of the hamsters are shown in Table 2. The animals fed the HCD for 30 d gained significantly more body and liver weight than those fed the SD. However, the body weight gains were significantly lower in the HPH and HPPS groups than in